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PATTERN OF CERTAIN ENZYME ACTIVITIES IN SERUM AND URINE IN DIFFERENT TYPES AND STAGES OF BILHARZIAL PATIENTS

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SUMMARY

The activity patterns of certain enzymes in serum and urine of 116 male subjects infested with S. haematobium or S. mansoni or both mixed (S. haematobium and S. mansoni in comparison to controls. were studied.

The material of study consisted of 116 adult male subjects infested with bilharziasis and proved to have no treatment for about 6 months prior to investigation.

In mansoniasis, the increments in serum GOT and serum GPT activities were more pronounced than in haematobiasis. Furthermor greater percentage of cases, showed higher serum GOT activity with the advancement of the disease, such a finding was not observed for serum GPT. Values for urinary GOT activity were within the normal range in patients with mansoniasis even when serum GOT activity as highly elevated (i.e. in the hepatosplenomegalic stage without ascites), hence ruling out its escape from serum into urine in such cases.

In cases of pure haematobium there is very high sigificant increase in serum GOT with a slight significant increase in serum GPT activity. In concomitant there appeared a high significant elevation in rinary GOT activity suggesting the renal system as its source and the release of its enzyme content into the urine.

In mixed infection, the changes in the activities of both serum GOT and GPT in the different stages did not show parallel changes when compared with the corresponding different stages of pure mansoniasis.

The elevation in serum GPT activity ran parallel with but to lesser magnitude than that for serum GOT activity in the different stages of mansoniasis. Thus, the abnormalities in the activities of serum GOT have been suggested as helpful guides to assess the extent of hepatic injury in mansoniasis, and when considered in conjunction with urinary enzymes that are known to be of renal origin (e.g. GOT) may reflect the extent of the involvement and damage of the kideys or renal tissues in the haematobiasis and mixed ifection, and may prove to be helpful as a laboratory tool in confirming the concomitant infection with haematobiasis (beside mansoniasis) even when one fails to discover living haematobium ova in the urine of mixed in fection..

The change of alkaline phosphatase (ALP) in pure mansoniasis, avery high significant increase in serum ALP activity was demonstrated in the uncomplicated stage of the disease. The participation of organs other than the liver particularly the intestine is suggested with longer duration and progress of the disease, serum ALP activities showed more sigificat elevations. Yet, no significant change in urinary ALP could be detected even in patients showing the highest serum ALP activity reflecting normal glomerular filtration function of the kidney.

In cases with haematobiasis, serum ALP activity showed high significant elevation as well as a very high significant increase in urinary ALP. However, no correlation could be observed betwee the extent of increase in urinary ALP activity and serum ALP in individual cases.

In cases with mxied infection, serum ALP activity was significantly elevated and the extent of elevation in the various stages exceeded that in pure mansoniasis. Besides, urinary ALP activity showed very high significant increase.

These results suggest that mansoniasis by itself causes high elevation in serum ALP activity, but when combined with haematobiasis there is additional efflux of renal tissue ALP into the circulation as well as the partial leakage of the latter into the urine as evidenced from the abnormally high levels demonstrated in either mixed infected or pure haematobiasis patients.

The results of isocitrate dehydogenase (ICD) demonstrate normal or non-significant slight changes in serum ICD activit in haematobiasis as well as the different stages of either pure mansoniasis or mixed infection (with the exception of the late stage of the latter cases).

In cases with pure mansoniasis, no significant elevation in serum Gamma-glutamyl transferase (γ -GT) could be observed in cases showing the 1st or 2nd stage of the disease, yet it was highly significantly increased in the 3red and 4th stages with further progress of the disease. the activity of uriary γ-GT was within the normal range.

In cases with haematobiasis, serum γ -GT showed no significant increments, yet this was accompanied with a very high significant elevation in urinary γ -GT activity suggesting the renal origin of the enzyme released in the urine.

In cases with mixed infection, no significt elevation of serum γ -GT could be demonstrated in cases showing the 1st stage of the disease. However, with the progress of the disease (i.e. 2 nd 3rd and 4th stages) Serum γ -GT activity was elevated to a extent that exceeded than observed in pure mansoniasis. Besides the mixed infected cases showed very high significant increase in uinary γ -GT activity asupporting our suggestion that the observed increment in urinary γ -GT activity is of renal origin.

In five mansonians, the different stages of the disease showed significant increase in serum Leucine aminopeptidase (LAP) activity. In cases of haematobiasis, serum LAP activity was slight significantly raised. In cases with mixed infection the different stage of the disease showed significant increase in serum LAP activity and the extent of such increase was parallel with the advancement of the disease.

From the choice and evaluation of the activities of a battary of enzymes particularly of specific to the organ in serum and/or urine may reflect the extent of hepatic and/or renal involvement and have greater advantages the evaluation of the activity of a single enzyme though the latter may be present in abundance in the target organ.

INTRODUCTION

It is well known that tissue destruction results in the release of some of its contents, including enzymes, into the accessible channel. It is well documented that the liver is the most and main important source for serum isocitrate dehydrogenase (**Okumura et al., 1960**) and the liver as well as the kidney are very rich in alkaline phosphatase (**Wachstein, 1958 and Butterworth and Moss 1966**), alanine aminotransferase and aspartate aminotransferase (**Wrobleski et al., 1956 and Dubach et al., 1966**), glutamyltransferase (**Rosalkie t al., 1973 and Teesdal et al., 1976**) and leucine aminopeptidase (**Wachstein, 1959**) and these organs could be involved in the causation of abnormalities of serum and/or urine enzyme patterns when involved in a disease.

In S. mansoni the liver could be considered as the site mainly and seriously affected and that the extet of damage is more dramatic with longer duration of the disease and its advancement from a stage to the next severe one. On the other hand, in haematobiasis the renal system including the kidneys is the system that is extensively invaded and involved, and such involvement may interfere with the normal handling of blood circulating proteins and enzymes though to variable degrees, depending upon the extent and type of involvement. Consequently, one can expect to find variable changes int he urinary enzyme pattern (e.g. γ -GT, GOT, GPT, ALP) that migh throw some light on the extent of renal involvement in urinary bilharziasis.

Thus, evaluation of the activities of these enzymes in both serum and urine may prove to be of value in unfolding the extent of organ derangements that accompany bilharziasis either pure mansoniasis, pure haematobiasis or both types mixed, and that may prove to be of value in the detection and/or identification of the type of bilhazial infection or a particular stage of the disease.

The present study includes the estimation of the activities of a battery of enzymes (ICD, LAP, γ -GT, ALP, GOT, GPT) that are known to be preset in abundance in liver and kidney tessues and that are known to have essential functions in a number of key metabolic proceses in the body.

The choice of a battery of enzymes has greater advantages than evaluation of the activity of a single enzyme though the latter may be present in abundance in the target organ. For example, ALP is present in the liver and its level in serum is known to be invariably increased in most hepatobilliary disorders (**Roealki, 1976**), yet it is hard to rule out the interference of other tissue ALP (e.g. bone, intestine... ect) in the causation of such rise is serum ALP.

The demonstration of a parallel rise in the activities of other serum enzymes that are known to be more related to the liver than other organs would support the conclusion of hepatic involvement as

the cause of the observed rise in serum ALP. In this connection, determination of serum GOT and γ -GT may serve as confirmatory indices for hepatic derangement to be the source for the rise observed in serum ALP.

MATERIAL AND METHODS

The study comprised 116 bitharzial adult male patients with age range, 18-65 years. None of the selected individuals was complaining from other diseases and proved to have no treatment for bitharziasis prior to their inclusion in this study. Data for age, weight and degree of liver and/or spleen enlargement were registered. Besides the bilharzial cases, 15 healthy adult normal individuals (Group C) with the same age range were includes in the study to serve as controls. The bitharzial cases were:

a- 15 patients with haematobiasis (Group H).
b. 74 subjects of mansoniasis were further subdivided into 4 subgroups:

I) 23 cases in the early stage of the disease where the liver was not enlarged and the spleen was not palpable (ml).

II) 21 subjects in the 2nd stage of the disease (m2). The main clinical feature was the occurrence of hepatomegaly, the spleen was not enlarged.

III) 15 cases in the 3rd stage of the disease (m3). This subgroup represented the hepatosplenomegalic stage of bilharziasis without ascites.

IV) 15 subjects in the 4th stage of the disease (m4), hepatosplenomegaly with ascites.

c- 27 cases with mixed Schistosomiasis were subdivided into 4 subgroups:

I) 14 Cases of mixed infection in early stage of mansoniasis (Mix_1)

II) 5 cases with hepatosplenomegalic stage of the disease (Mix_2)

III) 4 bilharzial subjects presented the hepatosplenomegaly without ascites (Mix_3)

IV) 4 patients with hepatosplenomegaly and ascites (Mix_4).

Before the initiation of any treatment, fasting blood samples were collected from patients using disposable syrings, and serum separated by centrifugation for 10 min. at 3000 r.p.m. Morning urine samples were collected and analysed immediately.

Determination of serum isocitrate dehydrogenase activity (ICD) were detrmined by the method of King (1967). Serum leucine aminopeptidase activity (LAP) were determined by methods described by Martinek et al. (1964). Colorimetric procedure for determination of serum and urine Gamma-glutamyl transferase activity were described by method of Naftalin et al. (1969 a). Aspartate transaminase and alanine transaminase activities were determined by Reitman and Frankel (1957).

RESULTS AND DISCUSSION

1. Serum Enzymes:

The data obtained for investigated serum enzymatic activities in our group of 15 healthy adult males used as controls revealed considerable agreement with those reported by other investigators. The units of ICD LAP and γ -GT are U/L. Attention should be given to the fact that certain enzymatic measurements are influenced in clinically healthy individuals by diet, race, sex, age, geographical distribution as well as other environmental factors (Copeland 1069). Study of alterations in a number of serum enzymatic activities is useful in the diagnosis of various types of liver disease because it may raflect many aspects of hepatic dysfunction (Harper, 1973) i.e. the extent of structural and functional damage in the liver.

The results for serum transaminases in haematobiasis revealed very high significant increase in aspartate aminotransferase (GOT) in concomitan

Table (1): Data for serum enzymatic activities in the group of 15 controls (group C), 15 cases with haematobiasis (group H) and 23 cases in the early stage of mansoniasis (first stage, m_1).

Group		ICD U/ml	LAP U/ml	γ-GT U/ml	ALP U/L	GOT U/L	GPT U/L	COT/GPT
Group (C)	Range	30-155	0.45-2.10	1.5-26.5	11.69-44.25	9.6-18.24	9,12-13.92	0.69-1.79
	M±S.E.	104±10.5	1.15±0.11	9.67±2.11	32.12±2.21	13.65±0.69	11.46±0.38	1.22±0.07
Group (H)	Range	35-180	0.5-2.2	1-29	24.21-67.64	12.48-39.36	10.56-24.96	0.81-2.0
	M±S.E.	93±12.36	1,51±0.14	10.17±2.15	44.53±3.07	20.36±1.72	14.14±1.18	1.47±0.09
	S_c	n.s.d.	s.s.i.	n.s.i.	h.s.i.	v.h.s.i.	s.s.i.	s.s.i.
First stage m_1.	Range	60-305	1.15-3.75	1-31	30.69-66.8	14.12-67.2	11.52-65.34	0.82-1.61
	M±S.E.	29.13±12.08	2.19±0.14	13.91±1.79	48.25±2.4	30.61±2.73	25.14±2.61	1.29±0.06
	S_c	n.s.i.	v.h.s.i.	n.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	n.s.i.
	S_H	n.s.i.	h.s.i.	n.s.i.	n.s.i.	h.s.i.	h.s.i.	n.s.d.

M± S.E. = Mean ± standard error.
S_c = Significance comparison to control.
S_H = Significance comparison to haematobiasis.

with a slight significant increase in alanine aminotransferase (GPT). These two variations resulted in a slight significant increase in the serum COT/GPT ratio, Since most of hepatic GOT is present in the mitochondria (Rej, 1978), and the GPT is cytoplasmic (Shoukry et al., 1972), it seems difficult to deduce that this increment in serum GOT is totally of hepatic origin. Since Schistosomiasis has been reported to cause damage, although to variable extent, to almost every organ in human body particularly when repeated infection occurs (Amador et al., 1963) it is possible that GOT from other organs most probably heart, skeletal muscles and kidney (Whitby et al., 1984 and Wroblewski, 1960). The participation of the kidneys is substantiated from finding of high urinary GOT content in our group of urinary bilharziasis.

On the other hand, in mansoniasis, where liver is more involved and with increased hepatic cells disintegration, the increment in both serum GOT and GPT were more pronounced than in haematobiasis, serum GOT showed elevated values in a great percentage of cases with advancement of the stage of the disease. Thus it showed elevated values in 78, 86 and 100% of cases in the 1st, 2nd and 3rd stages of the disease respectively, while for cases in the 4th stage only 80% of the cases revealed high values. However, the increase in serum GOT was highly significantly pronounced in the 4th stage of the disease than either the 1st, 2nd and 3rd stages of the disease respectively, while for cases in the 4th stage on 80% of the cases revealed high values. However, the increase in serum GOT was highly significantly pronounced in the 4th stage of the disease than either the 1st, 2nd

Table(2): Data for serum enzymatic activities in 21 cases in the hepatomegaly stage of mansoniasis (second stage, m_2), 15 cases in hepatosplenomegaly stage of mansoniasis with ascites(third stage, m_3) and 15 cases in hepatosplenomegaly stage of mansoniasis with ascites(fourth stage m_4).

Group		ICD U/mL.	LAP U/mL.	Y-GT U/mL.	ALP U/L.	SGOT U/L.	SGPT U/L	SGOT/SGPT
Second stage m_2	Range	35-365	1.15-4.10	0.5-114.5	24.21-101.87	12.48-64.32	10.56-49.92	0.82-2.15
	M±S.E.	32.14±14.75	2.26±0.17	21.48±5.76	52.45±4.50	30.63±3.28	22.01±2.30	1.43±0.07
	S_c	n.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i	s.s.i.
	S_H	n.s.i.	h.s.i.	v.h.s.i.	n.s.i.	m.s.i.	m.s.i.	n.s.d.
	S_{m_1}	n.s.i.	n.s.d.	h.s.i.	n.s.i.	n.s.i.	n.s.d.	n.s.i.
Third stage m_3	Range	35-500	1.2-5.00	10-126	29.22-129.42	20.16-50.88	12.48-26.92	1.10-3.53
	M±S.E.	31.33±14.09	2.51±0.26	39.07±7.72	68.36±7.60	30.45±2.30	17.15±1.16	1.85±0.17
	S_c	n.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.
	S_H	n.s.i.	h.s.i.	v.h.s.i.	h.s.i.	v.h.s.i.	n.s.i.	n.s.i.
	S_{m_1}	n.s.i.	n.s.i.	v.h.s.i.	h.s.i.	n.s.d.	s.s.d.	v.h.s.i.
	S_{m_2}	n.s.d.	n.s.i.	n.s.i.	n.s.i.	n.s.d.	n.s.d.	m.s.i.
Fourth stage m_4	Range	30-235	0.5-5.60	14-115	25.88-168.67	14.4-81.6	11.50-38.40	1.0-3.04
	M±S.E.	114±14.99	2.15±0.32	46.7±8.05	80.34±9.49	38.45±5.01	23±2.50	1.7±0.14
	S_c	n.s.i.	h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	h.s.i.
	S_H	n.s.i.	n.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	h.s.i.	n.s.i.
	S_{m_1}	n.s.d.	n.s.d.	v.h.s.i.	v.h.s.i.	n.s.i.	n.s.d.	h.s.i.
	S_{m_2}	n.s.d.	n.s.i.	h.s.i.	h.s.i.	n.s.i.	n.s.i.	n.s.i.
	S_{m_3}	n.s.d.	n.s.d.	n.s.i.	n.s.i.	n.s.i.	s.s.i.	n.s.d.

S_{m_2} = Significance in comparison to 2nd stage of mansoniasis.

S_{m_3} = Significance in comparison to 3rd stage of-mansoniasis.

Table(3): Data for serum enzymatic activities in 14 cases with mixed infection (haematobium and first stage of mansoniasis, mix_1), 5 cases(haematobium and hepatomegaly stage of mansoniasis, mix_2).

Group		ICD U/mL	LAP U/mL	Y-GT U/mL	ALP U/L	GOT U/L	GPT U/L	GOT/GPT
Mix_1	Range	20-295	0.7-5.55	0.5-28	15.03-107.71	13.44-75.376	12-64.32	1.08-1.79
	M±S.E.	11.79±12.02	2.13±0.32	12.18±2.30	59.64±7.87	28.01± 4.26	20.09±3.66	1.44±0.07
	S_C	n.s.i.	h.s.i.	n.s.i.	v.h.s.i.	h.s.i.	s.s.i.	s.s.i.
	S_H	n.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.d.
	S_{m_1}	n.s.d.	n.s.d.	n.s.d.	n.s.i.	n.s.d.	n.s.d.	n.s.i.
Mix_2	Range	60-155	1.50-4.90	3-61	56.8-87.7	17.28-30.92	12.96-26.88	0.82-1.77
	M±S.E.	95±16.05	2.59±0.60	23±10.12	75.84±5.99	26.19±2.77	20.16±2.42	1.36±0.17
	S_C	n.s.d.	v.h.s.i.	s.s.i.	v.h.s.i	v.h.s.i.	v.h.s.i.	n.s.i.
	S_H	n.s.d.	m.s.i.	n.s.i.	v.h.s.i.	n.s.i.	s.s.i.	n.s.d.
	S_{m_2}	n.s.d.	n.s.d.	n.s.i.	s.s.i.	n.s.i.	n.s.d.	n.s.d.
	S_{mix_1}	n.s.d.	n.s.d.	n.s.i.	n.s.i.	n.s.d.	n.s.i.	n.s.d.

S mix_1 = Significance in comparison to 1st stage of mixed infection.

or 3rd stages of the disease. This enormous elevation in serum GOT in the 4th stage of the disease is most probably due to the active process of hepatic cell necrosis in the late stage of the disease. Yet, it is noticed that 3 cases of the 4th stage revealed serum GOT values within the normal upper range and could be accounted for by :- (a) these cases could be in more advanced late stage, where the involved necrosed hepatic tissue is exhausted, and the residual reminant of the affected cells could not cause high elevation for this enzyme in the circulation, and/or (b) beside the cells being exhausted, a part of the enzyme in the circulation is lost into the ascitic fluid by diffusion through the serous membrane as do the plasma proteins (Wroblewski 1960). The latter postulate receives strong support from the findigns of Khattab et al. (1967), El-Hawary et al. (1970) and Shoukry et al. (1972) that showed a high serum protein content including enzymes into the ascitic fluid in cirrhotic ascitic bilharzial patients.

Regarding serum GPT in cases of mansoniasis, high values could be demonstrated in 83, 67, 67 and 67% of the cases of the 1st, 2nd, 3rd and 4th stages respectively (Table 6). Thus, it seems that serum GOT shows more correlation with the stages of the disease i.e. the extent of hepatic involvement than serum GPT. Such a finding lend support to the statement given by Wroblewski (1958) that "despite the greater activity of GPT in liver tissue, it seems that it is not specific for liver dis-

Table(4): Data for serum enzymatic activities in 4 cases with mixed infection (haematobium and hepatosplenomegaly stage of mansoniasis without ascites, mix_3), and 4 cases with mixed infection (haematobium and hepatosplenomegaly stage of mansoniasis with ascites mix_4).

Group	S.NO.	ICD U/mL	LAP U/mL	γ-GT U/mL	ALP U/L	SGOT U/L	SGPT U/L	SGOT/SGPT
Mix_3	Range	85-185	1.85-7.20	12.5-75.00	85.1-80.0	24.96-25.76	9.60-16.80	1.49-2.10
	M±S.E.	100±28.50	4.53±1.16	38.50±13.90	75.95±03.84	27.36±1.06	13.20±07.68	2.20±0.35
	S_c	n.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	n.s.i.	v.h.s.i.
	S_H	n.s.i.	v.h.s.i.	h.s.i.	v.h.s.i.	n.s.i.	n.s.i.	n.s.i.
	S_{a_3}	n.s.i.	h.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.
	S_{mix_1}	n.s.i.	h.s.i.	h.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.
	S_{mix_2}	n.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.	n.s.i.
Mix_4	Range	145-215	4.4-7.0	11.5-133.0	85.1-135.7	80.48-85.04	20.64-87.36	1.09-2.93
	M±S.E.	180±16.48	5.70±0.87	72.25±25.55	95.68±14.80	77.76±8.15	54.00±14.22	1.73±0.41
	S_c	h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	s.s.i
	S_H	h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	v.h.s.i.	n.s.i.
	S_{a_4}	s.s.i.	v.h.s.i.	n.s.i.	n.s.i.	v.h.s.i.	v.h.s.i.	n.s.i.
	S_{mix_1}	n.s.i.	v.h.s.i.	v.h.s.i.	s.s.i.	v.h.s.i.	h.s.i.	n.s.i.
	S_{mix_2}	n.s.i.	v.h.s.i.	n.s.i.	n.s.i.	v.h.s.i.	s.s.i.	n.s.i.
	S_{mix_3}	n.s.i.	n.s.i.	n.s.i.	n.s.i.	v.h.s.i.	s.s.i.	n.s.i.

S_{mix_2} = Significance in comparison to 2nd stage of infection.

S_{mix_3} = Significance in comparison to 3rd stage of mixed infection.

Table (5): Data for urine enzymatic activities in the group of 6 controls, 9 hepatosplenomegaly mansoniasis without ascites (m_3), 12 haematobium cases and 4 mixed infection in the hepatosplenomegaly without ascites (mix_3).

Group		γ-GT U/mL	ALP U/L	GOT U/L
Group (C)	Range	0.00-0.04	1.00-3.34	2.50-3.99
	M±S.E.	0.02±0.01	2.37±0.33	3.32±0.23
Third stage m_3.	Range	0.02-0.04	1.34-2.34	2.88-3.65
	M±S.E.	0.03±0.003	1.99±0.11	3.32±0.10
	S_c	n.s.	n.s.	n.s.
Group (H)	Range	0.06-0.46	3.34-7.58	4.03-7.68
	M±S.E.	0.23±0.04	4.75±0.33	4.15±0.32
	S_c	v.h.s.i.	v.h.s.i.	v.s.h.i.
	S_{m_3}	v.h.s.i.	v.h.s.i.	v.s.h.i.
Mix_3	Range	0.29-0.38	6.49-9.85	6.19-9.45
	M±S.E.	0.31±0.03	8.11±0.70	7.78±0.69
	S_c	v.h.s.i.	v.h.s.i.	v.h.s.i.
	S_{m_3}	v.h.s.i.	v.h.s.i.	v.h.s.i.
	S_H	n.s.i.	v.h.s.i.	v.h.s.i.

ease, and that elevation of serum GPT can be used as a part of a battery of enzymes to establish whether liver damage has occurred.

In cases of mixed infection, there is still significant increments in both serum GOT and GPT. The increase in serum GOT was observed in 79, 80, 100 and 100% of the cases in the 1st, 2nd, 3rd and 4th stages of the disease as referred to mansoniasis complications. However, the extent of the rise was somewhat less than in the corresponding stages of pure mansoniasis.

Of interest to mention, is that the results for serum GPT in the different groups of bilharziasis included in the present study ran parallel with thsoe for serum GOT, although the extent of rise in GPT was a lesser magnitude than that for GOT especially in cases of the third and fourth stages of the disease, as illustrated by the high rise in GOT/GPT ratio in these latter two stages of the disease. This could be accounted for by the fact that more than 81% of hepatic cells GOT is located in the mitochondria (Rej, 1978) while most of the liver tissue GPT exist as a soluble cytoplasmic enzyme (Murray, 1987) and hence on damage of hepatic cells at greater rate in the advanced stages (i.e 3rd and 4th stages) of the disease the amount of GOT efflux in the circulation will be at a higher magnitude leading to the demonstrated greater incre-

Table (6): Summary for the percentage of patients with abnormal serum high enzymatic activities (higher than the upper normal limit for each enzyme activity).

Group	No. of cases.	ICD	LAP	Y-GT	ALP	GOT	GPT	GOT/GPT
Haematobiasis	15	20%	13%	7 %	53%	60%	20%	13%
Mansoniasis :								
First stage of mansoniasis (m_1)	23	26%	43%	0.0%	61%	78%	83%	4 %
Second stage of mansoniasis (m_2)	21	24%	62%	24%	62%	85%	67%	5 %
Third stage of mansoniasis (m_3)	15	20%	53%	40%	73%	100%	67%	47%
Fourth stage of mansoniasis (m_4)	15	27%	40%	53%	93%	80%	67%	33%
Mixed schistosomiasis:								
Haematobiasis and m_1	14	14%	29%	0.0%	53%	79%	57%	0.0%
Haematobiasis and m_2	5	0.0%	60%	20%	100%	80%	80%	0.0%
Haematobiasis and m_3	4	25%	75%	50%	100%	100%	50%	75%
Haematobiasis and m_4	4	75%	100%	75%	100%	100%	100%	25%

N.B.: The percentage is calculated to the nearest total figure.

ments in the GOT/GPT ratio. This suggestion receives strong evidence from the extent of increase in GOT/GPT ratio in the different stages of mansoniasis which proved to be n.s.i, s.s.i, v.h.s.i and h.s.i. in the 1st, 2nd, 3rd and 4th stages of mansoniasis when compared to controls, respectively. Of interest to add is that the relative decrease from v.h.s.i. in the 3rd stage to h.s.i. in the 4th stage of the disease may be due to variation in the rate of loss of serum GOT and GPT into the ascitic fluid in the latter stages of the disease.

The rise in serum transaminases activities in bilharziasis particularly mansoniasis could be mainly attributed to hepatic involvement:

i) Increased liver cell damage under the effects of bilharzial worms, eggs or the toxins elaborated by them (Snay, 1957), Higazi, et al. (1960), Saif et al. (1964), Saleh et al. (1964), Ghanem et al. (1970; a + b), Adul-Nasr et al. (1973), El-Haieg et al. (1978, a + b).

ii) A hypothetical cell membrane defect of submicroscopic dimensions leading to parenchymal hepatic damage (El-Gholmy et al., 1969) with increased hepatic cell membrane permeability (Snay, 1957) and subsequent leakage or seepage of hepatic cytoplasmic enzymes e.g. GPT, into the blood.

In the advanced cases of bilharzial hepatic fibrosis (Higazi, 1960, a + b; Ghanem, 1970, c), particularly those with ascites (Ghanem et al., 1970) and the finding of Mansour et al. (1965) that myopathy may occur in mice infected with S.mansoni.

The demonstration of abnormal high levels fo serum GOT and GPT in our groups of different types of schistosomiasis (urinary, intestinal or mxied) supports similar studies carried out on humans (Higazi et al., 1960, Saif et al., 1964) and experimental animals (Garson et al., 1957; Sadun, and Williams 1966).

Yet, there seems an apparent conflict between our findings for serum GPT and those reported by El-Haieg et al. (1978) and Sadun and Williams (1966) who reported that the rise in serum GPT was relatively greater than of GOT and with those published by other investigators (Salah, 1962; Pautrizel et al., 1963 and Murray, 1987) who reported GPT levels to be within normal in their groups of bilhrziasis.

Of value to add, is that Sommerville et al. (1960) found that serum GOT activity in patients with decompensated cirrhosis was significantly higher that in those with compensated cirrhosis; while serum GPT activity was not significantly altered by the development of decompensation.

On the bases of out findings and those reported by other investigators, it is possible to conclude that the changes in serum GOT and GPT may serve as helpful guides to assess the extent of hepatic injury in mansoniasis, and when considered in conjunction with urinary enzymes (e.g. GOT) may reflect the extent of kidney or renal tissue damage in both haematobiasis and mixed infestation. To our knowledge and according to the available literature, no similar trials have been undertaken to correlate the enzymatic activities in serum and urine in such groups of bilharzial patients.

The data obtained for serum alkaline phosphatase in haematobiasis revealed values exceeding the upper normal limit in 53% of the cases. It is well established that serum alkaline phosphatase activity primarily reflects changes in bone and liver function, thouh high alkaline phosphatase activity can be detected in other organs (Pankow et al., 1972). Furthermore, any drug or element that interferes with hepatic function or is hepatic toxic or induces cholestasis could cause an increase in serum alkaline phosphatase, sometimed dramatically.

In early stages of mansoniasis and mixed infection there is also a significant rise in serum alkaline phosphatase. This supports the findings of Mansour et al. (1982) who reported elevated serum alkaline phosphatase in cases of S. mansoni infection with no complication. Of interest to mention, is that Fishman et al. (1965) demonstrated an increase in serum alkaline phosphatase of intestinal origin in patients with liver disease. Since S. mansoni infestation is a disease which primarily affects the intestine beside other organs. It is important to add that the alkaline phosphatase is considered a very sensitive indicator of liver diseases when principally affect parenchymal cells, a

condition which is known to occur since the early stages of mansoniasis (Hashem et al., 1947). A very high serum alkaline phosphatase in cases of liver cirrhosis whether due to viral infection or chronic advanced bilharziasis (Diechoff et al., 1964). Also, it is parallel to similar reports by other investigators in mice (Awadalla et al., 1974; Awadalla et al., 1975 and Gareeb et al., 1975) and in monkeys (Bersohn and Lurie 1953) infected with S. mansoni and in human patients showing bilharzial hepatic fibrosis (GLanem et al., 1979; e + f and El-Haleg et al., 1978, b).

The pattern of serum alkaline phosphatase among cases with mixed infection simulates that discussed for pure mansoniasis. However, the extent of elevation of the various stages exceeded in the former those demonstrated in the latter. Since the kidneys are one of the richest organs in alkaline phosphatase, it is possible to propose that such increase in the extent of rise may be due to the additional efflux of renal tissue alkaline phosphatase into the circulation. Such a view receives support from the finding of elevated alkaline phosphatase activity in the urine of mixed infection as well as haematobiasis.

Serum levels of leucine aminopeptidase (LAP) and gamma-glutamyl transferase (γ-GT) activities appear to parallel and approximate the degree of elevation of the hepatic isoenzyme of ALP values in hepatobiliary disease but not in osseous diseases (Lum & Gambino, 1972; and Rosalki, 1976).

Isocitrate dehydrogenase (ICD) has in fact been advocated as a highly specific and very sensitive indicator for the diagnosis of parenchymal acute hepatic damage (Strekat et al., 1963 and Wilkinson, 1976) and can be seen in the early incubation phase of the disease. Also the enzyme has been shown to be particularly sensitive in detecting minor episodes of hepatic damage due to alcohol (Goldberg, 1975) and drugs (Wates et al., 1969 and Young, 1975).

In cases with mixed infection, high significant increase in serum ICD activities was observed in the late stage of the disease i.e. in advanced prominent hepatosplenomegaly with ascites.

Gamma-glutamyl transferase (γ-GT) in serum has a M.Wt. of 90,000 daltons, as measured by electrophoresis (Tsuhi et al., 1980) and appears originate primarily from the liver (Huseby, 19 The chief clinical value of measuring γ -GT is enzymatic sensitivity as indicator of hepatobili disease since it is elevated in all form of liver d cases.

In recent years, the enzyme assay of γ -GT leve serum has been used to suggest and detect the t sue origin of observed serum ALP elevation (Z et al., 1970 and Shoukry et al ., 1972), since e vation of γ-GT activity is in parallel excess w the degree of ALP increase in liver disease on whereas ALP may be increased without γ-GT e vation in bone, cerain renal and gastrointestina diseases. Concomitant liver and bone disease can not be excluded when both enzymes a raised, but is less likely if a high γ-GT accompa ies slight ALPelevation (Lum et al., 1972).

The results of the present study show that the a tivity of serum γ-GT in cases of haematobiasis a well as in early stages of mansoniasis (all cases o the 1st stage beside those of the 2nd stage excep cases) as well as in the early uncomplicated 1s stage infestation, were not significantly elevate This supports the finding of normal serum γ-G activity reported by Mansour et al. (1982) for h group of patients in the early uncomplicated stag of mansoniasis. The afore-mentioned four case asigned clinically as belonging to the 2nd stage o the disease and demonstrated elevated serum γ GT activity may be clinically on the border line t enter in the 3rd stage of the disease where serum γ-GT values were elevated in a greater percentag of the cases (40%).

In comparison with the results for serum ALP i these different stages of the disease, it can be no ticed that serum ALP showed elevated values in a greater percentage of cases than serum γ-GT These differences seem to add some support t our suggestion that the observed elevated serum ALP in haematobiasis as well as in the early stag es of mansoniasis and mixed infection may not b totally of hepatic origin but it might at least par tially be of intestinal origin.

With the progress of the disease to the stages o hepatosplenomegaly with and without ascites, the

extent of elevation in serum γ--GT is more pronounced. Besides, such elevation is manifested in a greater percentage of cases. In addition, the elevation in cases with mixed infection far exceeded that in cases with pure mansoniasis, and this difference can be attributed to the release of renal γ--GT into the circulation in mixed infection cases as the kidney which is very rich in this enzyme is involved under the effect of haematobiasis which is a part of the mixed infection form of the disease. This postulate is also supported by the finding of elevated γ- -GT activity in urine of cases suffered from mixed schistosomal infection as well as pure haematobiasis, while being normal in cases suffering from pure mansoniasis.

γ--GT is a membrane-bound microsomal enzyme and its tissue level increases in response to enhanced microsomal enzyme synthesis (Szewezuk, 1966 and Zimmermann 1984) Furthermore, the hepatic enzyme has been demonstrated histochemically and biochemically (Glenner et al., 1962 Albert et al., 1964, Aronsen et al., 1969 and Naftalin et al., 1969;b) in the canaliculi of the parenchyma, and especially in the luminal border of the epithelial cells lining the fine biliary ductules. So, the elevation of serum γ--GT activity may be due to the release of the enzyme from the membrane matrix to which it is normally anchored (Rosalki, 1975 and Goldberg, 1980). This suggestion in confirmed and supported by the finding that activity of γ--GT within the peripheral hepatic cells (Glenner et al., 1962; and Albert et al., 1964) has been observed and that this γ--GT activity may be increased by inflammation or cirrhosis (Gibinski et al., 1963). In parallel, it can be postulated that enhanced microsomal γ--GT enzyme synthesis may take place under the effect of the bilharzial worms or their eggs or toxins elaborated by them.

Leucine aminopeptidase (LAP) is another liver enzyme that has relatively broad specificity Rutenberg et al. (1964) indicated that in other diseases not involving the hepatobiliary tract or pancrease, LAP was only occasionally elevated. However, Shay et al. (1960) reported that neither the pancrease itself nor carcinoma of the pancrease is responsible for elevation in serum LAP. Thus the increase of serum level of LAP activity is most probably a specific and sensitive indicator of various diseases of hepatobiliary system (Arst et al., 1959; Bressler et al., 1960; Merikas & Anganostous, 1964) and liver damage (Bardawil et al., 1972). Further reports indicate that serum values for LAP activity are generally normal in patients with bone diseases (Rutenburg et al., 1964; Ardwalll, 1965 and Zimmermann et al., 1984). Sasz and Balogh (1965) concluded that measurements of LAP activity can be useful in differential diagnosis of several liver and biliary diseases in infants. These findings had led to the suggestion that distinction between osseous and hepatobiliary disease as a cause of elevated serum ALP levels can be provided by assay of serum LAP activity.

The results obtained in the present study show that patients infected with bilharzial mansoni and presented the early uncomplicated stage (m_1) hepatomegaly stage (m_2), hepatosplenomegaly stage without ascites (m_3), or hepatosplenomegaly stage with ascites (m_4) demonstrate a significant increase in serum LAP activity as compared to controls. These data support the results obtained by Khafagy et al. (1976), though the latter authors did not report about the serum LAP in the early uncomplicated stage of mansoniasis (m_1) and those reported by D'onoferio et al. (1964) who showed raised serum LAP in hepatomegalic ascitic cirrhosis and in splenomegalic cirrhosis. But cases of hepatosplenomegaly with ascites (m_4) showed values lower than cases of hepatosplenomealy without ascites (m_3): this may be due partly to: (a) the ascape of some of the enzyme to the ascitic fluid since Fleisher et al. (1959) found the enzyme to be present in the ascitic fluid in patients with liver cirrhosis though in considerably lower activity than that of the serum, and (b) partly due to encreased elimination of the enzyme in urine.

In mixed infection cases, the results show that serum LAP activity also increased significantly in the different stages of the disease, and that such increment was parallel with the advancement of the disease. However, cases in the most advanced stage of mixed infection (haematobium and hepatosplenomegaly with ascites, mix_4) showed the highest serum LAP activity that exceeded the cor-

responding stage in pure mansoniasis (m_4) This greater rise could be accounted for by the additional release of the enzyme from the involved kidneys (Glenner, 1962) under the effect of haematobiasis as a part of the mixed schistosomal infection type of the disease. This postulate receives support from the data obtained for serum LAP in our group of urinary bilharzial patients where the results showed a slight significant increase in the serum enzyme activity. Increased of interest to add, is that LAP is widely distributed in certain human tissues, among which is the small intestine where it is abundant (Rutenberg et al., 1958). Serum LAP level in the early uncomplicated stages of either mansoniasis or mixed infection may be partly due to the escape of certain amounts of intestinal LAP into the circulation under the effect of mansoni passing ova which cause damage to intestinal wall.

Infiltration of the intestines and the portal tracts of the liver with bilharzial granulation tissue (which is formed of the bilharzial ova in the centre surrounded by epithelial giant and oesinophil cells and changes in liver cells due to deposition of this granulation tissue may result in increased liver cell damage or increased membrane permeability of hepatic parenchyma and this may be the cause of the release of LAP from the hepatic cells since liver cell necrosis in bilharziasis does not occur except very terminally (Khafagy et al., 1976).

Of interest to mention, is that for the most part serum LAP values may provide some additional information that is not completely available when other liver enzyme analyses are used (Ellis et al., 1978), therefore serum LAP determination has enjoyed some popularity in studies concerned with primary and secondary hepatic disorders.

(II) Urinary Enzymes:

When a tissue or an organ is involved in a disease process and the permeability of its cells is affected and increased, or if its cells are disintegrated or damaged, its contents including the enzymes are released into the accessible channels. For the internal organs, e.g. liver, heart,...etc., the easily and directly accessible channel is the blood circulation, hence the release of enzymes from such organ tissues would be reflected as an increase in the enzyme activity in the blood. Haematobiasis (particularly in the active state and stage of passing living ova) is usually accompanied with different degrees of haematuria. The latter could aid or participate in the causation of an elevation of urinary enzymatic content as a result of loss of certain amount of the serum enzymes that is present in the circulation.

These points gave us the impetus to carry out investigation on the levels of a number of enzymes that are known to be present in considerable amounts in the kidney (namely γ-GT, ALP and GOT) in different groups of our patients.

Gamma-glutamyl transferase:

In human tissue, the highest γ-GT activity is prominent in the kidney (Goldberg et al., 1960) with its highest activity in the renal tubules, where it is 12 times higher than in the pancreas and 2 times higher than in the liver. Low activities are also, found in the small intestine, the spleen, and other organs.

Despite high activity in kidney (Szasz, 1974), renal diseases may not be accompanied or accompanied with slight increases in γ-GT activities in the serum (Rosalki, 1975 and Schimdt et al., 1973). Yet, elevated urinary activity were reported in acute renal disorder and in nephrosis. Besides the properties of γ-GT in such urines, and in normal urine, and the urinary γ-GT isoenzyme pattern are identical to those seen in the renal tissue but differ from those of the γ-GT of plasma (Jacyszyn & Laursen, 1968 and Szasz, 1970).

In our group of haematobiasis, urine showed very highly significant elevation of γ-GT level, while that of hepatosplenomegaly stage of mansoniasis without ascites showed urinary γ-GT level within the normal range. Our previous results concerning serum γ-GT level in haematobiasis revealed slight but non-significant elevation, while that of hepatosplenomegaly stage of mansoniasis without ascites showed highly significant increase. Briefly in hepatosplenomegaly stage of mansoniasis without ascites, γ- GT level was elevated in serum but not in urine, while in haematobiasis γ -GT level

was elevated in urine but not in serum. Therefore, our results of the present study suggest that the elevation of urinary γ-GT in hasematobiasis is mainly derived from the kidney and seems not, or only partially , derived from the liver through pouring of its enzymes into the circulation. This suggestion supports the results obtained by Orlowski (1963), Lehmann et al., (1970) and Szasz (1970) that revealed urinary γ-GT levels within the normal limits elevation of the plasma enzyme in patients with liver disease; in other words, in such cases urinary γ-GT did not appear to result from excretion of the plasma enzyme, since plasma and urine enzyme levels showed no correlation .

Of interest, haematobiasis has been reported to be accompanied with very high significant increase in total urinary amino acids among adult males and children (El-Hawary et al., 1975), and such loss was attributed mainly to renal involvement and haematuria. It has been proposed that γ-GT of the renal proximat tubular cells is important to conserve amino acids because γ-GT participates in the reabsorption of amino acids from the glomerular filtrate by the formation of γ--glutamyl peptides, which are absorbed into, and then broken down by renal cells, with return of their component amino acids to the blood (Orlowski, 1963). The loss of certain amounts of the γ-GT enzyme in urine from renal tissues under the effect of worms, eggs or their toxins could lead to dysfunction in the mechanism of amino acids reabsorption from glomerular filtrate, and hence participates in the causation of the aminoaciduria observed in heaematobiasis as well as possibly other renal disorders.

In mixed infection cases, where the above deteriorating effect of haematobiasis is present, and with longer duration of the disease the glomerular filtration function may be involved leading to loss of certain quantities of the elevated serum enzymes-arising under the effect of the concomitant mansoniasis into the urine. This is in accordance with the finding of different degrees of proteinuria in patients suffering from haematobiasis (Abdin, 1974), and receives substantial evidence from our results that showed very highly significant increments in urinary γ-GT in mixed infection cases than in cases with pure haematobiasis.

Alkaline Phosphatase:

Under normal coditions, very low ALP activity could be detected in human urines, and it seems to be derived from the kidneys (Butterworth, 1966, Butterworth et al., 1965). In contrast to serum, no isoenzymes of ALP in urine have been detected (Wajima, 1963). Biochemical and histochemical investigations revealed the presence of high activities of ALP in renal tubular cells (Wachstein, 1955 and Wacker, et al., 1961) and it has been shown that brush borders contain high levels of ALP (Novikoff, 1959).

The values for ALP activity achieved in the present study show that urinary ALP activity in hepatosplenomegaly stage of mansoniasis without ascites was within the normal limit with no significant change from controls. This finding reflects the normal glomerular filtration function in such cases. On the other hand, urinary ALP activity in haematobiasis and in mixed infection cases was very highly significantly elevated as compared to controls. However, the extent of increment in urinary ALP activity did not correlate with the extent of increase in the activity of the corresponding serum ALP

Experimental investigations on changes of urinary ALP have been performed in different species of experimental animals (Ascher & Anson, 1960, Bergmann & Truss, 1964 and raab, 1966), and showed that chemical tubular damage and renal ischemia increased urinary ALP (Bergmann et al., 1964 and Harper, 1973). Also, many renal diseases have been shown to increase urinary ALP activity: acute and subacute nephritis, renal lupus erythematosis, tubular necrosis, acute renal failure, renal infarction and tumors of the kidney as well as of the bladder (Amador et al., 1963, 1965, Butterworth et al., 1963, Dubach et al., 1966; a + b).

Based on our observations, it could be suggested that the increase in urinary ALP activity in haematobiasis is mainly of renal origin, and in mixed infection mansoniasis by itself causes high elevation in serum ALP and with the possibility of abnormalities in the glomerular filtration function,

the serum enzyme rise may at least partially (through its leakage) participate in the causation of elevation in urinary ALP activity.

Glutamic-Oxaloacetic Transaminase:

Under normal conditions, only very low GOT activity is present in human urine (Amelung et al., 1956 and 1958). GOT activity has been demonstrated in urines without any detectable proteinuria (Pletschmann et al., 1960).

In all species examined, high activities of GOT was found in renal tissue (Dieckhoff et al., 1964, a and Dubach, 1963). Human kidney tissue has been reported to have GOT in moderate amounts which are relatively concentrated in the glomerulus (Wroblewski, 1958). The presence of GOT in the urine of normal people is probably due to the result of normal "wear and tear" breakdown of cells in the urinary tract.

The results for GOT activity achieved in our present study show that both serum and urinary GOT activities were elevated in haematobiasis either when pure or mixed with mansoniasis, while in hepatosplenomegaly stage of pure mansoniasis without ascites only serum GOT activity was elevated. Since S. haematobium worms, eggs or its toxins affect the kidney besides other regions of the renal system, the above results strongly suggest that the increase in urinary GOT level is most probably of renal origin.

From the above studies concerning the urinary enzymes it could be stated that assay of certain enzyme activities-that are known to be of renal origin in the urine of haematobiasis could throw some light on the extent of involvement of the renal system in such cases. Besides, it may prove to be helpful as a laboratory tool in confirming the concomitant infection with haematobiasis (beside mansoniasis) even when one fails to discover living haematobium ova in the urine of mixed infection cases.

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